Evaluation of Antidepressant Activity and the Possible Mechanism of Action of *Majoon Najah* in Experimental Models

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Abstract

The present study has been carried out to evaluate the antidepressant activity of a pharmacopoeal Unani drug *Majoon Najah* (MN) in experimental animals. Tetrabenazine antagonism test and Yohimbine toxicity enhancement test were used to study the antidepressant activity in mice divided into 4 groups of 6 animals each. Animals in Group I, II and III were treated with distilled water, 50% alcoholic extract of MN in 260 mg/kg (single dose) and 520 mg/kg (double dose), orally, respectively. Group IV was treated with standard drugs Imipramine (20 mg/kg per oral) and Desipramine-Hcl (10 mg/kg i.p.) in both the tests, respectively. The effect of test drug was observed on duration of catalepsy, degree of ptosis and the mortality rate of the animals.

MN demonstrated antagonist effect in Tetrabenazine induced catalepsy and ptosis. Cataleptic score and degree of ptosis were significantly reduced ($p<0.001$) in Group II and III in a dose dependent manner, and no significant difference was found between Group III and IV. In Yohimbine toxicity enhancement test, the mortality rate increased significantly ($p<0.001$) in Group II & III; and at 24 hr significant difference was observed when mortality rate was compared among the groups, between I & III ($p<0.011$), and between I & IV ($p<0.05$), between II & III ($p< 0.011$). The mean time of mortality in group III was observed significantly less ($p<0.0001$) when compared with group I, II & IV.

The study demonstrated that the test drug possesses significant anti depressant. It has most likely produced its effect by inhibiting the monoamine uptake through adrenergic, serotonergic and monoamine oxidase inhibiting mechanisms.

Keywords: Antidepressant, *Majoon Najah*, Yohimbine, Catalepsy.

Introduction

Depression is a disorder of emotion rather than disturbance of thought. Major depression which affects approximately 20% of the population is classified as either unipolar or bipolar (Porth and Kunert, 2002). It is characterized by a state of low mood and aversion to activity that can affect a person's thoughts, behavior, feelings and physical well-being and is twice common in women than in men (Salman, 1997). Although, the currently prescribed molecules have shown signs of improvement in the clinical condition of the patients, but it is at the cost of having to bear the burden of their numerous adverse effects and chances of recurrence (Stahl, 1998).

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In Unani system of medicine the term "*Malikholia*" (Melancholia) is commonly used for depression. Melancholia is one of the often used words of psychiatry, while depression is the recent name of melancholia (Rao, 2004). Hippocrates (460-357 BC) described melancholia as a state of "aversion to food, despondency, sleeplessness, irritability and restlessness" (Kaplan and Sadock, 1995). It is defined as a disease in which there is derangement of thoughts and intellect. It is characterized by social isolation, loneliness, fear of objects an average person is not afraid of, negative thoughts and feelings, excessive grief, anxiety, delusions, hallucinations etc. The disease has been described to be caused mainly due to disproportionate (excessive) accumulation of black bile or deterioration in its quality (Jurjani, 1898; Tabri 1995; Garzooni, 1994; Ibn Sina, 2007; Razi, 2002). The symptoms of *Malikholia* as described in Unani literature, are withdrawal from the society, negative thoughts and feelings, inability to think and act rationally, excessive grief, hallucinations, delusions, feeling of worthlessness or excessive guilt, fearfulness without a cause, nervous exhaustion, sleeplessness, restlessness, loss of interest and enjoyment, fatigue and loss of energy etc (Jurjani, 1898; Ibn Sina, 2007). These symptoms have similarity with the symptoms of depression described in DSM-IV (Anonymous, 1994). Therefore, *Malikholia* has been taken by us to correspond to the depressive disorder. There are a number of drugs both single and compound preparations that are used in Unani medicine in depressive disorders since hundreds of years. One important pharmacopoel compound drug is Majoon Najah (MN) described in all major formulary books of Unani medicine. It is a semi-solid preparation obtained by mixing different powdered drugs as mentioned below (Table 1), in a *qiwam* (base) made of purified honey or sugar.It is an age-old and time tested polyherbal preparation which is commonly used in depression and related conditions (Kabiruddin, 1938). MN has also been investigated on scientific parameters and shown to be significant antidepressant, CNS stimulant, anxiolytic and antioxidant activities (Imran, 2008) using Gross Behaviour Test, Despair Swim Test, Reserpine Induced Hypothermia Test, Pentobarbitone Induced Narcosis Potentiating Test and Elevated plus Maze Tests etc. The present study was designed with an aim to assess the antidepressant effect of the test drug and also to explore the possible mechanism of action especially with reference to monoamine concentration. In depression, since there is a deficiency of neurotransmitters noradrenaline and serotonin in the brain, which can be altered by antidepressants therefore the drugs that effect depression, can modify amine storage release or uptake. In view of the above therefore two important tests i.e. Tetrabenazine antagonism test and Yohimbine toxicity enhancement test were used to determine its anti depressant effect and the likely mechanism of action. However, the extract of the ingredients sans sugar/honey was used.

Table 1: Ingredients of Majoon Najah

Ingredients	Parts used	Weight
Post Halila Kabli (Pericarp of *Terminalia chebula* Retz.)	Pericarp	50 g
Post Balela (Peel of *Terminalia belerica* Roxb.)	Pericarp	50 g
Amla Khushk (Fruit of *Emblica officinalis* Gaertn.)	Fruit	50 g
Halila Siyah (Unripe Fruit of *Terminalia chebula* Retz.)	Unripe Fruit	50 g
Turbud Mudabbar (Root & stem of *Ipomoea turpethum* Br.)	Root /stem	25 g
Bisfaij (Rhizome of *Polypodium vulgare* Linn.)	Rhizome	25 g
Aftimoon (Whole plant of *Cuscuta reflexa* Roxb.)	Whole plant	25 g
Ustukhuddus (Whole plant *Lavandula stoechas* Linn.)	Inflorescence	25 g

Materials and Methods

The present study was undertaken in the department of Ilmul Advia, National Institute of Unani Medicine (NIUM) Bangalore, Karnataka, India. Before starting the experiment the protocol was submitted to the Institutional Animal Ethics Committee (IAEC) of NIUM Bangalore, for ethical clearance. The proposal was approved vide Reg No. IAEC/V/05/IA dated 25/04/2010.

Plant Drug Material

All the ingredients of MN were procured from the local market of Bangalore. Professor Amthul Shukoor (Senior Botanist and Taxonomist, University of Mysore) authenticated the drug samples (vide letter No.D.Auth-01/2010-11 dated 25-06-2010). The specimens of the ingredients have been submitted to NIUM herbarium library for record and future reference.

Preparation of Extract

The dried plant materials were pulverized separately and the coarse powder obtained was mixed and stocked in plastic containers from which extracts were prepared. 100 g of powdered drug was extracted separately in 400 ml of ethyl alcohol (50%) along with water (50%) in Soxhlet apparatus at a temperature of 70°-80°C for 8 hours continuously. The extract obtained from each sample was filtered, cooled and evaporated on water bath till it dried. It was weighed and the yield percentage was calculated with reference to the crude drug. The average yield of the hydro- alcoholic extract of three samples of MN was found to be 39%.

Animals

The study was carried out in two different groups of male Swiss mice with one group weighing between 20-22 gm for Tetrabenazine Antagonism test and the other weighing between 25-28 gm for Yohimbine Toxicity Enhancement Test (Vogel, 2002). The mice were procured from Central Animal Research facility (CARF), National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. They were housed in polypropylene cages (6 animals per cage) and were maintained under standard laboratory conditions with temperature at 25 ± 2^0 c, relative humidity of 50%-60 % and 12 hours light/dark cycle at the animal house facility of NIUM. Mice were given standard pellet diet (Lipton-India ltd.) and tap water ad *libitum* under strict supervision and hygienic conditions.

Dosage of Drug

The dose of the Hydro alcoholic extract of MN for Swiss mice was calculated by multiplying the therapeutic dose of the test drug as describe in Unani literature, by conversion factor 12 (Frierich et al., 1968) and found to be 260 mg/kg. To evaluate the dose dependent response the double dose i.e. 520 mg/kg was also used in the study.

1. Tetrabenazine antagonism test

This test was carried out by the method of Vogel (2002). Swiss male mice weighing between 20-22 gm were used in this test. The animals were observed for catalepsy and ptosis induced by TBZ. The mice were divided into four groups of six animals each and treated per orally as follows:

Group-I: Control group was administered Distilled water 0.25 ml.

Group-II: Treated with MN in the dose of 260 mg/kg.

Group-III: Treated with MN in the dose of 520 mg/kg.

Group-IV: Treated with standard drug Imipramine in the dose of 20 mg/kg.

All the drugs and the vehicle were administered once in the morning. The time of administration of treatment was recorded. Sixty minutes after the administration of treatment, Tetrabenazine (TBZ) was mixed with a drop of glacial acetic acid and diluted with 0.9% saline and was administered in the dose of 40 mg/kg intraperitoneally, to all the animals in each group (Yamada, 1994; Fabio, 1999). 30 minute after the administration of TBZ, animals of all the groups were observed for Catalepsy individually. Each mouse was placed on a cork stair which was made of two cork stoppers having 2 steps of 3cm height each on which the animals were placed head downwards with their hind legs upon the top cork. Cataleptic effect was observed as long as TBZ exerts its cataleptic effect. The

duration of catatonic state of each mouse was recorded with the help of stop clock. Cataleptic effect was observed for a maximum of 60 seconds. Scores were allotted depending upon the duration of catalepsy produced in each and every mouse. Scoring pattern was adopted as follows:

Duration of catalepsy	Scores
>60 sec	5
Between 30 - 60 sec	4
Between 10 - 30 sec	3
Between 05 - 10 sec	1
<05 sec	0

In the above experiment TBZ – control were taken as 100%. Whereas standard drug Imipramine has shown its effect at a dose of 20 mg orally.

Soon after observation of catalepsy Mice were placed into normal position and placed into cages. After a gap of just 30 seconds the animals were again observed for ptosis which was produced due to the effect of TBZ administration. Ptosis was observed at an interval of 30 minutes up to a maximum of 150 minutes and the degree of ptosis was recorded in each and every mouse. The pattern of awarding the degree of ptosis was adopted as follows:

Eyes close ..... 4^0

Eyes ¾ closed ..... 3^0

Eyes ½ closed ..... 2^0

Eyes ¼ closed ..... 1^0

Eyes open ..... 0^0

2. Yohimbine Toxicity Enhancement Test

This test was carried out by the method of Vogel (2002). Swiss male mice of body weight between 25 to 28 g were used for this test. They were divided into four groups of ten animals each. The mice in group I were treated as a negative control with 0.25 ml of distilled water orally. Mice in group II were treated with MN in the dose of 260 mg/kg orally and group III were given MN in the dose of 520 mg/kg orally while group IV were treated with standard drug Desipramine-HCL at the dose of 10 mg/kg i.p. All the drugs and the vehicle were administered once in the morning 30 minutes before the conduction of test.

Exactly after 30 minutes of administration of test drug, a sub lethal dose of 25 mg/kg of Yohimbine-HCL was given, subcutaneously. Yohimbine-HCL occupies central α_2 receptor and prevents noradrenaline from binding to these receptors.

An anti depressant is known to inhibit physiological inactivation of noradrenaline and other biogenic amines by blocking the re-uptake at nerve terminal. Administration of antidepressant (Standard and the test drug) leads to an increase in noradrenaline concentration. Following the simultaneous administration of Yohimbine and an antidepressant, deaths of mice have been recorded due to noradrenaline poisoning which has exhibited the antidepressant activity of the drugs.

Mortality rate was assessed at every 1, 2, 3, 4, and 24 hrs. Lethality in Yohimbine negative control group has been mentioned as less than 10% and about 90% in standard drug of Desipramine HCL at the dose of 10 mg/kg. Death rate was also recorded by giving in two different doses of the test drug. This test has been proven as simple and critical assessment method to detect antidepressants with monoamine uptake inhibiting properties.

Statistical Analysis

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. Kruskal Wallis test a non-parametric test has been used to find the significance of study parameters between three or more groups of animals, Kaplan Meir Function analysis is performed to find the significance of time to death in each group. Mann Whitney U test has been performed to find the pair wise significance. Fisher Exact test has been used to find the significance incidence of death in four different groups.

Observations and Results

Effect of MN on Tetrabenazine Induced Catalepsy and Ptosis

Catalepsy

The cataleptic effect in the mice was observed for a maximum of 60 seconds at a regular interval of 30, 60, 90, 120 and 150 min (max). The duration of catalepsy was recorded; the mean and median scores of catalepsy were obtained from the experimental data and were compared among the different groups by Kruskal Wallis test (Table 2).

During the first observation after 30 minutes, the mean and median cataleptic score in Group II was found to be 3 as compared to Group I which was 4.67. This shows that the cataleptic score was significantly reduced by 1.68 (Z=3.146). The mean and median cataleptic score of Group III was found to be 1 when

Table 2: Comparison of Mean Catalepsy Score in Four Groups

Catalepsy	After 30 min	At 60 min	At 90 min	At 120 min	At 150 min
Group I	4.67(5.00)	4.67(5.00)	4.67(5.00)	4.67(5.00)	4.67(5.00)
Group II	3.00(3.00)	3.00(3.00)	3.00(3.00)	3.00(3.00)	3.00(3.00)
Group III	1.00(1.00)	1.00(1.00)	1.00(1.00)	1.00(1.00)	1.00(1.00)
Group IV	1.00(1.00)	1.00(1.00)	1.00(1.00)	1.00(1.00)	1.00(1.00)
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Pair wise difference					
Group I vs Group II	1.68	1.68	1.68	1.68	1.68
Group I vs Group III	3.67	3.67	3.67	3.67	3.67
Group I vs Group IV	3.67	3.67	3.67	3.67	3.67
Group II vs Group III	2.00	2.00	2.00	2.00	2.00
Group II vs Group IV	2.00	2.00	2.00	2.00	2.00
Group III vs Group IV	0.00	0.00	0.00	0.00	0.00
Pair wise Comparison (Z values)					
Group I vs Group II	3.146**	3.146**	3.146**	3.146**	3.146**
Group I vs Group III	3.146**	3.146**	3.146**	3.146**	3.146**
Group I vs Group IV	3.146**	3.146**	3.146**	3.146**	3.146**
Group II vs Group III	3.317**	3.317**	3.317**	3.317**	3.317**
Group II vs Group IV	3.317**	3.317**	3.317**	3.317**	3.317**
Group III vs Group IV	0.00(NS)	0.00(NS)	0.00(NS)	0.00(NS)	0.00(NS)

Results are presented as Mean; Z- value is obtained by Kruskal Wallis test

compared with Group I, the cataleptic score was found to be decreased by 3.67 (Z=3.146), which was even more significant. When the mean and median cataleptic score were compared among the groups, it was found that, the cataleptic score in Group III was decreased significantly by (2.00) i.e. (Z=3.317). The cataleptic score in group- III and group IV were found to be similar and there was no statistically significant difference. This shows that the double dose of test drug and the standard drug have similar effect.

Similarly mean and median cataleptic score in group - II, at 60, 90, 120 and 150 min interval were found to be 3, 3, 3 and 3 when compare with group -I which were found to be 4.67, 4.67, 4.67, 4.67 respectively. When the mean and

median cataleptic scores were observed at 60, 90, 120 and 150 min interval and compared , the cataleptic score in group III was decreased by 2.00, 2.00, 2.00 and 2.00 i.e. ($Z=3.317^{**}$ sig), which was highly significant when compared with group- II. The cataleptic score in group- III and group IV at 60, 90, 120 and 150 min interval were again found to be similar and there was no statistically significant difference between these two groups, shows that the double dose of test drug and the standard drug have similar effect.

Ptosis

The animals were observed for ptosis at a regular interval of 30, 60, 90, 120 and 150 min (max). The degree of ptosis was recorded and ranged from 0^0-4^0 in which higher degree indicates augmentation in ptosis and lower degree indicates reduction in the degree of ptosis.

The mean and median degree of ptosis was obtained from the experimental data using Mann Whitney-U test (Table 3) and the overall degree of ptosis was found to be 4^0 in Group I, 2^0 in Group II, 0^0 in Group III and 0^0 in Group IV. When the first observation for ptosis was done at 30 min, during the experiment the degree of ptosis in the Control Group I was found to be maximum i.e. 4.00, while in Group II degree of ptosis was 2.33. When the mean degree of ptosis was compared among different groups at 30 min, it was found that the mean degree of ptosis of Group II was significantly less 1.67 (Z=3.146) than Group I; the mean degree of ptosis in Group III was found to be 0 which was highly significant (Z=3.317) as compared to Group I. However, no significant difference was observed between Group III and IV. When Group II was compared with Group III, the mean degree of ptosis was found to be significantly reduced (2.33, Z=3.146).

Observation for ptosis was also done at 60, 90, 120 and 150 minutes and it was found that the mean degree of ptosis in Control Group was maximum i.e. 4^0 throughout the recording of the experiment. While in Group II degree of ptosis was 2, 2, 2 and 1, at 60, 90,120 and150 min, respectively. The mean degree of ptosis of Group II when compared with Group I was found to be significantly less by1.83 (Z= 3.108), 2.17 (Z= 3.207), 2.33 (Z= 3.146) and 2.67 (Z=3.146), at 60, 90, 120, and 150 minutes, respectively, throughout the experiment. The mean degree of ptosis in Group III was found to be 0, 0, 0 and 0, at 60, 90, 120, and 150 minutes, respectively, when it was compared with Group I, it was found less by 4 (Z=3.317) at all the intervals as there was no degree of ptosis observed in the animals of Group III and when it was compared with Group IV the degree of ptosis was statistically similar between Group III & Group IV, and when Group II was compared with Group III the mean degree of ptosis was significantly reduced

Table 3: Comparison of Mean and Median Degree Ptosis in Four Groups

Ptosis	After 30 min	At 60 min	At 90 min	At 120 min	At 150 min
Group I	4.00(4.0)	4.00(4.0)	4.00(4.0)	4.00(4.0)	4.00(4.0)
Group II	2.33(2.00)	1.83(2.00)	1.83(2.00)	1.67(2.00)	1.33(1.00)
Group III	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)
Group IV	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Pair wise difference					
Group I vs Group II	1.67	1.83	2.17	2.33	2.67
Group I vs Group III	4.00	4.00	4.00	4.00	4.00
Group I vs Group IV	4.00	4.00	4.00	4.00	4.00
Group II vs Group III	2.33	2.17	1.83	1.67	1.33
Group II vs Group IV	2.33	2.17	1.83	1.67	1.33
Group III vs Group IV	0.00	0.00	0.00	0.00	0.00
Pair wise Comparison (Z values)					
Group I vs Group II	3.146**	3.108**	3.207**	3.146**	3.146**
Group I vs Group III	3.317**	3.317**	3.317**	3.317**	3.317**
Group I vs Group IV	3.317**	3.317**	3.317**	3.317**	3.317**
Group II vs Group III	3.146**	3.108**	3.207**	3.146**	3.146**
Group II vs Group IV	3.146**	3.108**	3.207**	3.146**	3.146**
Group III vs Group IV	0.00(NS)	0.00(NS)	0.00(NS)	0.00(NS)	0.00(NS)

Results are presented in Mean; Z- values are obtained by Pair wise comparison done by Mann Whitney U test

by 2.17 (Z=3.108), 1.83 (Z=3.207), 1.67 (Z=3.146) and 1.33 (Z=3.146) at 60, 90, 120, and 150 minutes, respectively.

Effect of Majoon Najah on Yohimbine Toxicity Enhancement

The effect of intervention of test drug based on mortality was assessed at 1, 2, 3, 4, 5 and 24 hrs of study period (Table 4 and 6). During the observation, the mean mortality rate was compared among the different groups by Fisher Exact test.

Table 4: Comparison of Mortality Rate in Four Groups

Mortality	1 hour	2 hours	3 hours	4 hours	5 hours	24 hours
Group I						
• Alive	10 (100.0%)	10 (100.0%)	10 (100.0%)	10 (100.0%)	10 (100.0%)	9 (90.0%)
• Death	0	0	0	0	0	1 (10.0%)
Group II						
• Alive	10 (100.0%)	10 (100.0%)	10 (100.0%)	9 (90.0%)	7 (70.0%)	6 (60.0%)
• Death	0	0	0	1 (10.0%)	3 (30.0%)	4 (40.0%)
Group III						
• Alive	8 (80.0%)	5 (50.0%)	2 (20.0%)	1 (10.0%)	1 (10.0%)	1 (10.0%)
• Death	2 (20.0%)	5 (50.0%)	8 (80.0%)	9 (90.0%)	9 (90.0%)	9 (90.0%)
Group IV						
• Alive	9 (90.0%)	8 (80.0%)	4 (40.0%)	2 (20.0%)	2 (20.0%)	2 (20.0%)
• Death	1 (10.0%)	2 (20.0%)	6 (60.0%)	8 (80.0%)	8 (80.0%)	8 (80.0%)
P value	0.595	0.001	<0.001	<0.001	<0.001	<0.001

2x4 Fisher Exact test

Effect of M N on Yohimbine Toxicity Enhancement

At 1 hr, the mean mortality rate of Swiss mice was found to be 0%, 0%, 20% and 10% in group I, II, III, and IV, respectively, which when compared among different groups it was found that there was no significant difference between these groups (Table 5).

At 2nd hr the mean mortality rate was 0%, 0%, 50% and 20% in group I, II, III and IV, respectively, which on comparison with different groups demonstrated that there was no difference between Group I, II and IV. However, Group I and II when compared with group III showed significant difference ($p<0.05$), while significant difference was observed between group III and IV.

Table 5: Comparison of Rate of Death in Four Groups

N=10	Incidence of Mortality			
	Group I	Group II	Group III	Group IV
1 hour	0	0	2(20.0%)	1(10.0%)
2 hours	0 c^1	0 a^4, c^1	5(50.0%) a^1,b^1,d^4	2(20.0%) a^4, c^4
3 hours	0 b^4,c^3	0 a^4, c^3	8(80.0%) a^3,b^3, d^4	6(60.0%) a^1,b^1 c^4
4 hours	0 c^3	1(10.0%) c^3, d^2	9(90.0%) a^3,b^3, d^4	8(80.0%) a^3,b^2 c^4
5 hours	0 c^3	3(30.0%) c^1	9(90.0%) a^3,b^1, d^4	8(80.0%) a^3 b^4 c^4
24 hours	1(10.0%) b^4,c^3, d^2	4(40.0%) a^4,c^3	9(90.0%) a^3,b^3, d^4	8(80.0%) a^2, b^4

(P value is obtained by Fisher Exact test, n = 10, mean ± SD (Min - Max) and results on categorized measurements are presented in number %)

1, 2, 3 & 4 = p < 0.005, p < 0.011, p d" 0.001 & N.S (Not significant)

a = Comparison with group -I (Control)

b = Comparison with group-II (Test drug A)

c = Comparison with group- III (Test drug B)

d = Comparison with group-IV (Standard drug)

At 3rd hr the mean mortality rate was 0%, 0%, 80%and 60% in group I, II, III, and IV, respectively. Significant difference was found when Group I and II were compared with Group III (p< 0.001), whereas Group I and II when compared with Group IV showed a significant difference (p<0.011). However, no significant difference was observed between Group I and II as well as between III and IV.

At 4th hr the mean mortality rate was 0%, 10%, 90% and 80% in group I, II, III, and IV, respectively. During inter group comparison the values of Group I were found significant (P <0.001) as compared to Group III and IV. Group III and IV (P<0.001) and Group II and IV (P<0.005) also demonstrated significant difference when compared with each other. No significant difference was found between Group III and IV.

At 5th hr the mean mortality rate was 0%, 30%, 90% and 80% in group I, II, III, and IV, respectively. Significant difference was found when Group I was compared with Group III and IV (P< 0.001); Group II showed significant difference (P<0.05)

Table 6: Prediction of the Time of Mortality (Kaplan Meir Function Test)

	Mean time of death	SE	95%CI
Group I	>24.00	0.0	-
Group II	18.20	3.23	11.86-24.54
Group III	4.50	2.07	0.43-8.56
Group IV	7.10	2.69	1.84-12.36
Inference	Time of death in hrs is significantly early in Group III (4.50 hrs), followed by Group IV (7.10 hrs) (P<0.0001) (Log rank test)		

when compared with Group III. No significant difference was observed between III and IV group.

At the end of the study i.e. at 24 hr the mean mortality rate was 10%, 40%, 90% and 80% in group I, II, III, and IV, respectively. A significant difference was observed between Group I and Group III, Group I and IV (P<0.001) and between Group II and III (P<0.001). However, there was no significant difference between Group I & II, group II & IV and group III & IV.

Kaplan Meir function test was performed to assess the mean time of mortality. The findings summarized in Table 6 indicate that the mean time of mortality in Group III was 4.5 hours which was significantly less than the mean time of Group I and II (P<0.0001). In Group I, the mean time of mortality was found to be more than 24.00 hrs and in group II, it was 18.20 hrs. However, the mean time of death in Group IV was found to be slightly higher i.e. 7.1 hours than Group III. Therefore, the early onset of deaths in group III when compared to other groups suggested that the double dose of test drug has better response.

Discussion

In the present study, hydro alcoholic extract of MN was evaluated for antidepressant activity on two experimental models of depression. These two tests are considered simple and reliable for the evaluation of classical antidepressant drug through which both Monoamin oxidase inhibitory (MAOIs) and Tricyclic antidepressant (TCAs) effects may be evaluated (Vogel, 2002). The findings of Tetrabenazine antagonism test suggested that there was a significant reduction of catalepsy and ptosis. In this experiment, the test drug ameliorated the catalepsy and ptosis caused by TBZ through noradrenergic, serotonergic and monoamine oxidase inhibition as the TBZ induces depletion of biogenic amines (eg. noradrenaline, serotonin and dopamine) from nerve terminals without affecting their de novo synthesis and prolongs reuptake into the granula.

Noradrenaline is degraded by monoamine oxidase, this depletion of monoamine actually produces catalepsy and ptosis (Vogel, 2002).

It has been reported that all clinically useful antidepressant drug potentiate, either directly or indirectly, the action of norepinephrine, dopamine and/or serotonin in the brain (Mary *et al*., 2000). The standard TCA drug Imipramine which was used in this test is a strong reuptake inhibitor of norepinephrine and serotonin (http://drugbank) and acts as an adrenergic and seratonergic (Fabio et al., 1999). It inhibits the reuptake of noradrenaline into nerve terminals and thereby increases its concentration at the receptor site (Vogel, 2002). By decreasing the degree of catalepsy and ptosis the test drug appears to antagonize the effect of TBZ. When the results were compared with that of control group, the two doses of test drug were found to reduce the cataleptic score of ptosis significantly ($p<0.001$), in dose dependent manner as the effect of double dose was found to be more significant than the single dose, while no significant difference was observed between the results of Group III Group IV. Therefore, the findings suggested that the test drug possesses striking antidepressant effect that is equable to standard drug Imipramine.

The findings of Yohimbine toxicity enhancement test suggested that there was a significant increase in the mean mortality rate of test drug. In this experiment, Yohimbine-Hcl occupies central α_2 receptors and prevents noradrenaline from binding to these receptors, thus allowing an increase in noradrenaline concentration. It has been reported that an anti depressant drug inhibits physiological inactivation of noradrenaline and other biogenic amines by blocking the reuptake at nerve terminals and consequently increasing the biogenic amines concentration (Mary, 2000). Desipramine which was used as the standard drug in this test is known to exhibits greater non adrenergic reuptake inhibition as compared to other TCAs (Fabio, 1999). Therefore, following the simultaneous administration of Yohimbine and an antidepressant, death of mice was recorded due to noradrenaline poisoning. Here the mechanism involves dual activity both by blocking the selective reuptake of noradrenaline from the neural synapse in the CNS by using an antidepressant and also by administration of Yohimbine which lead to high concentration of noradrenaline resulting in death of mice. When the results were compared with that of control group, the mortality rate was found significantly increased ($p<0.001$) at single and double dose of MN. At 24 hrs, when the mortality was compared among different groups it showed that the more number of animals died in less time after treatment with double dose of the test drug. This observation revealed that the test drug increased noradrenaline and other mono amine concentration by the similar mechanism as that of standard drug Desipramine-Hcl. This test has proved the antidepressant activity of MN via adrenergic reuptake inhibition, in a dose

dependant pattern. The findings of present study in respect of its anti depressant effect are in agreement with the findings of previous study (Imran, 2008).

Almost all the ingredients of the test drugs are described in Unani literature to possess *Munzije Sauda* (concoctive of black bile) and *Mushile Sauda* (purgative of black bile) properties, therefore they are able to improve a diseased condition where the *sauda* is accumulated in excessive amount or its quality is compromised, giving rise to certain pathological conditions. Since depression as discussed earlier, is mainly caused by the qualitative or quantitative imbalance of *sauda,* the improvement in depressive condition by the test drug therefore suggests that it possesses antidepressant effect because of its *Munzije Sauda/ Mushile Sauda* properties. *Munzij* and *Mushil* properties are mainly responsible to improve depression by removing the causative factor or improving its quality. Thus, the claim of Unani medicine that the drugs possess *Munzije Sauda/Mushile Sauda* activity can be used in the management of depressive disorders, has been validated in this study.

Conclusion

In view the findings of present study it can be concluded that *Majoon Najah* possesses significant antidepressant effect. It increases the concentration of noradrenaline at the receptor site probably through adrenergic reuptake inhibition and blocking the degradation of noradrenaline.

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